BRITISH MEDICAL BULLETIN

Vol. 1. (1943) No. 5

The object of this Bulletin is to provide a guide to medical science and thought in Britain, and it consists mainly of summaries of a representative selection of British papers on subjects of medical interest. Any material appearing in the Bulletin may be published without fee, but acknowledgment of the source, by addition of the initial letters BMB followed by the serial numbers of the items selected, would be appreciated. The Bulletin is not distributed generally to the medical profession.

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SPECIAL CONTRIBUTION

RECENT WORK ON BLOOD TRANSFUSION IN BRITAIN

by P. L. MOLLISON, M.B., M.R.C.P.

In the war of 1914–18 the conclusion had already been reached that the most effective single step in the treatment of severe wound shock was the restoration of blood volume by blood transfusion. Inevitably, therefore, great attention has been given to all aspects of this subject in the present war and knowledge has advanced very considerably.

Apparatus.—One of the most important if undramatic advances has been the introduction of a simple standardised apparatus for taking and giving blood by a closed method, that is to say, one in which the blood does not come into direct contact with the open air (*for description, see* Vaughan, 1939). Because of the standardisation there is complete interchangeability of apparatus from all parts of the country, whether produced by the civilian or military services.

Stored Blood.—Storage of blood was practised in only a few hospitals in Britain before the present war. In anticipation of the demands which very heavy air-raids might create, blood banks were established in London at the beginning of the war and in other parts of the country soon afterwards. At first, blood was stored in a simple citrate-saline solution, but soon the observation by Rous and Turner of the beneficial effect of the addition of glucose was confirmed (Harington & Miles, 1939; Maizels & Whittaker, 1940a; Dubash, Clegg & Vaughan, 1940) and a citrate-glucose mixture came into general use. At first, there was naturally much discussion as to the relative merits of stored and fresh blood. Early reports showed, however, that transfusions of stored blood did not give rise to more reactions than those of fresh blood (Stewart, 1940), and that the former were equally efficacious, at least in the treatment of acute hæmorrhage (Brewer, Maizels, Oliver & Vaughan, 1940).

It was realised that the exact value of stored blood could be most satisfactorily measured by estimating the survival rate of the erythrocytes after transfusion, and many workers reported that erythrocytes stored in citrate-glucose mixtures survive well in the recipient's circulation (Bushby, Kekwick, Marriott & Whitby, 1940; Maizels & Paterson, 1940; Mollison & Young, 1940). Many interesting observations were published upon the physical and chemical changes occurring during storage (Maizels & Whittaker, 1939, 1940a, 1940b; Crosbie & Scarborough, 1940, 1941, 1942; Scarborough & Thompson, 1940). Earlier work was confirmed when it was shown that the erythrocytes lose potassium during storage (Downman, Oliver & Young, 1940; Aylward, Mainwaring & Wilkinson, 1940). Maizels & Paterson showed, however, that some at least of these changes were reversible and that stored erythrocytes lost sodium again in the recipient's circulation after transfusion. Some confusion continued to exist as to the best preservative solution and Maizels (1941b) suggested that the laboratory test of measuring the osmotic fragility of stored erythrocytes might give a false indication of the way in which they would survive after transfusion. This point of view was supported by Mollison & Young (1941) who found that although red cells stored in the Rous-Turner solution became very fragile, they survived very well in the recipient's circulation. Conversely, red cells stored in sucrose became very resistant to hæmolysis by hypotonic saline but survived poorly in the recipient's circulation. These workers found that other *in vitro* tests were also misleading, and they therefore undertook a trial of several preservative solutions, using both *in vivo* and *in vitro* tests (Mollison & Young, 1942). They found that the Rous-Turner solution was the best, but considered that its large bulk was too great a disadvantage to warrant its use in preference to the ordinary small volume citrate-glucose solution.

All citrate-glucose solutions have one disadvantage, namely that the citrate and glucose solutions have to be autoclaved separately to prevent the occurrence of caramelisation. Evans, Thorley & McLeod (1942) showed, however, that if the mixture were acidified with carbon-dioxide before autoclaving, caramelisation was prevented. Later, Loutit, Mollison & Young (1943) found that this method was ineffective in their autoclaves, but observed that the addition of citric acid in suitable proportions not only diminished caramel formation but greatly improved the preservative properties of the solution as judged by the survival *in vivo* of the erythrocytes after transfusion. There seems to be little doubt that acidified citrate-glucose mixtures are the most satisfactory blood preservatives yet discovered.

Plasma and Serum.—The only blood substitute available in the last war with an osmotic pressure of the order of human plasma was gum saline, which was eventually shown to have serious disadvantages despite the good immediate results attending its use. Great attention has been given in the present war to the problems involved in using stored human plasma and serum as blood substitutes. In the beginning, the advantage of preparing plasma seemed obvious, since plasma could be obtained as a by-product from stored blood. After MacKay (1941) had shown that none of the available antiseptics could be relied upon to inhibit bacterial growth in liquid plasma in a concentration which would not be toxic if a large transfusion had to be given, it was recognised that plasma stored in the liquid state would have to be Seitz-filtered before storage. It was found, however, that plasma clotted spontaneously after being passed through a Seitz filter. This phenomenon was investigated by Macfarlane, Macsween, Mainwaring & Parish (1942), who showed that, when plasma was passed through a Seitz filter, fibrinogen and prothrombin were at first retained but were present in later samples of the filtrate. It was observed that these later samples were the first to exhibit spontaneous clotting. This clotting could only be prevented by limiting the amount of plasma passed through a given Seitz pad. Even then, its occurrence was not postponed indefinitely. Bushby, Buttle & Whitby (1940) showed that washing of the filter pad with alkali enabled a greater volume of plasma to be passed through one pad before clotting occurred, and this observation was utilised in devising a process for large-scale filtration of plasma. Later, the addition of alkali to the plasma before filtration was advocated (Bushby & Whitby, 1942). In this latter process, the pH was brought back to 7 by mixture with carbon dioxide under pressure. Clegg & Dible (1940) tackled the problem in a different way by calcifying the plasma and then filtering off the clot. Picken (1941) advocated the addition of serum to plasma as a method of precipitating the fibrin. Maizels (1941a) suggested the combination of the two methods in order to reduce the amount of calcium needed and to avoid the necessity for the addition of the large relative volume of serum required in the previous method.

Perhaps the most successful approach to the problem has been made by McFarlane (1942), who has devised a process for extracting the fibrinogen with ether at a low temperature; the resulting product remains clear for very long periods.

Apart from its freedom from fibrinogen, serum has the advantage over plasma that it is more suitable for drying, and since the beginning of the war serum has been dried by a special *Medical Research Council* Unit. In 1940, Adair, Adair & Greaves reported that the osmotic pressure and electric charge of the proteins of human serum were unaffected by the process of drying from the frozen state, and a clinical trial of serum dried in this way proved satisfactory (Brown & Mollison, 1940). The advantages of the dried product have become more and more apparent as experience of the liquid products has accumulated. The most obvious of the advantages are a far greater stability, ability to withstand a wide range of temperature variations, and freedom from the risk of supporting bacterial growths. The process of drying from the frozen state has lately been refined by spinning the bottles as they cool and by "snap-freezing" (Greaves, 1941). In this way an almost amorphous product is produced which has a far greater solubility than the earlier material. Harrison & Picken (1941) have advocated the use of defibrinated blood for transfusion since unwanted blood then directly yields serum.

Shock and Acute Hæmorrhage.—The first trial of plasma transfusions in combating shock in air-raid casualties proved

most successful (Kekwick, Maycock, Marriott & Whitby, 1941). Grant & Reeve (1941) concluded that, in severely injured patients who had lost much blood, the simple measures of rest, warmth and morphine administration were usually insufficient for recovery, but that a transfusion of blood or plasma was a most potent means of restoring or maintaining the circulation. They considered that whether or not there was appreciable hæmorrhage, the great majority of severely injured patients benefited by transfusion. Black (1940) reported good results from treating burns with intravenous plasma.

Volume and Rate of Transfusion.—The modern conception that the amount of blood administered to a patient with anæmia should bear a direct relationship to the degree of increase in hæmoglobin level required, owes much to Marriott and Kekwick. These authors have also urged the importance of administering transfusions at a drip rate. They have advocated (1940) that the rate of transfusions for the relief of anæmia should never exceed 1 cm.^{3} per pound [0·545 kg.] of body weight per hour, and should not exceed half this rate in subjects suffering from severe anæmia, or cardiac or respiratory disease. As emphasised by Whitby (1942), these rules were never intended to apply to transfusions given to accident cases for the restoration of blood volume, and in such cases rapid transfusion is advisable. Whitby mentioned that in 150 cases receiving massive transfusions no single instance of pulmonary œdema was observed. Grant & Reeve (1941) from an analysis of observations on 100 air-raid casualties concluded that early transfusions should be given at the rate of 500 cm.^{3} in 15 to 30 minutes and that later transfusions, unless given to combat further bleeding, should be administered more slowly, namely at the rate of 500 cm.^{3} in one or more hours.

The view that subjects with a normal cardiac muscle will tolerate extremely rapid transfusions was supported by observations made by Sharpey-Schafer & Wallace (1942). Thirteen convalescent subjects received from 700 to 2100 cm.^{3} of human serum intravenously at rates varying between 54 and 168 cm.^{3} per minute. Apart from a transient feeling of constriction in the chest, in some of the subjects, no symptoms or untoward results were observed. A temporary diminution in vital capacity with an increase in the density of the lung shadows and an increase in venous pressure were noted however. Loutit, Mollison & van der Walt (1942) noted that the rise in venous pressure following a transfusion depended rather upon the volume transfused than upon the rate of administration. Sharpey-Schafer & Wallace, whose experience was similar, noted that the rise in venous pressure depended upon the retention of the transfused fluid in the circulation.

Concentrated Red Cell Suspension.—When liquid plasma was first being prepared from stored blood, the possibility of using the residual erythrocytes was explored by MacQuaide & Mollison (1940). At first the erythrocytes were suspended in saline, but later concentrated suspensions were prepared by simply adding together the residues from two bottles of stored blood after removing the bulk of the plasma. Very satisfactory results have since been reported by many authors (Vaughan, 1941; Whitby, 1941; Davidson & Stewart, 1941; Williams & Davie, 1941) using these concentrated erythrocyte suspensions for the treatment of cases of anæmia. An important advantage of their use is the reduction in the total volume of fluid to be administered for the achievement of a given rise in hæmoglobin.

Blood Grouping.—As experience of mass blood grouping has accumulated the possible sources of error have received more attention and the necessity for the use of a reliable technique has been emphasised (War Memorandum No. 9, 1943).

Understanding of compatibility has undoubtedly made its greatest advance since the original discovery of the four blood groups, with Landsteiner and Wiener's discovery of the Rh agglutinogen. This agglutinogen is present in the majority of human bloods and its chief importance lies in the fact that it is capable of provoking the formation of specific immune agglutinins in persons (Rh-negative) whose erythrocytes lack the agglutinogen. The discovery by Levine that Rh-negative women may become sensitised to the Rh agglutinogen during pregnancy when their fœtus is Rh-positive, and the further discovery that the immune agglutinins formed in the mother's circulation may pass back across the placenta and cause *erythroblastosis fœtalis*, have added enormously to the importance of this new finding. These observations have been confirmed by Boorman, Dodd & Mollison (1942), who found that the incidence of the Rh agglutinogen in the English population is similar to that amongst American Whites. They also found, like Levine, that over 90% of the mothers of infants affected with *erythroblastosis fœtalis* are Rh-negative and they described cases in which persons who had become sensitised to the Rh agglutinogen had suffered severe hæmolytic reactions due to the transfusion of Rh-positive blood. Taylor, Race, Prior & Ikin (1942) have described some of the difficulties which may be encountered in making tests for Rh agglutinogens and agglutinins and in particular have drawn attention to the occurrence of zoning in certain anti-Rh sera.

Destruction of Erythrocytes in Vivo.—Application of the technique of differential agglutination, whereby the survival rate of transfused erythrocytes in the recipient's circulation can be estimated quantitatively, is likely to prove increasingly important in the solution of blood transfusion problems. As mentioned above, this method has already been used to decide the question of the value of various solutions for the storage of blood, and the same method is proving invaluable in investigating the new problem of intra-group incompatibility (Mollison, 1943). Transfused erythrocytes should not be eliminated from the recipient's circulation at a rate greater than approximately 1% per day. A study of the causes which lead to an increase in this rate should add much to knowledge of hæmatology in general.

The damage to the recipient's erythrocytes that can be caused by the transfusion of blood, the plasma of which contains high-titre incompatible agglutinins, was studied by Aubert, Boorman, Dodd & Loutit (1942). To simplify the problem, they used plasma of group O rather than blood of group O, and administered it intravenously to subjects of group A. They found that when the plasma contained very potent anti-A agglutinins, samples taken from the recipient immediately after transfusion might show hæmoglobinæmia or intravascular agglutination. Nevertheless, in no case did they produce a really serious reaction.

Summary of Progress.—Investigators in the field of blood transfusion may be said to have achieved important objectives during the present war. The apparatus has been so simplified that transfusions can be given under almost any circumstances. Stored blood can now be kept for periods of 3 weeks or more and still be virtually as efficacious as fresh blood from the point of view of restoring blood volume and of supplying functioning erythrocytes. Stable and satisfactory blood substitutes which can be stored for months or years have been provided. Finally, understanding of transfusion accidents and therefore of the means of preventing them has advanced very considerably.

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[1][*See BMB* **8**] [2][*See BMB* **94**] [3][*See BMB* **86**] [4][*See BMB* **5**] [5][*See BMB* **84**]

Dr. P. L. Mollison has been working since 1939 *at the South-West London Blood Supply Depôt, one of a number of similar Depôts which have been organised by the Medical Research Council for the Ministry of Health as part of a national blood transfusion service, which supplies the needs both of the civilian population and of the Armed Forces. Dr. Mollison has published, with other members of the medical staff of the Depôt at which he works, a number of papers on various aspects of blood transfusion.*

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THE DETERMINATION OF BLOOD GROUPS

Medical Research Council War Memorandum No. 9, 1943

This memorandum is issued under the authority of a Blood Transfusion Research Committee appointed by the *Medical Research Council*. The technical principles of blood group determination, which must be strictly adhered to if errors in blood grouping are to be avoided, are less widely known than they deserve to be. The objects of the Memorandum are (i) to describe methods which in trained hands have given satisfactory results, (ii) to recommend certain methods, and (iii) to analyse the possible sources of error inherent in all group determinations.

Principles of Diagnosis of the ABO Blood Groups

Two main agglutinogens A and B by their presence or absence in human erythrocytes determine the blood group; the corresponding agglutinins anti-A and anti-B are found in the appropriate human sera. Hæmagglutination occurs whenever homologous agglutinin and agglutinogen are in contact. Unknown erythrocytes are therefore tested for their blood group with known anti-A and anti-B sera. For the most reliable results, however, not only the erythrocytes but also the serum of the unknown are tested, the dual test providing a check on technical and clerical errors.

The Sub-Groups of A

Sub-groups A_1 and A_1B and A_2 and A_2B can be recognised by the fact that the cells of A_2 and particularly of A_2B bloods react more weakly than those of A_1 and A_1B with anti-A test sera. Nearly all anti-A sera from group B and group O donors contain the agglutinins alpha and $alpha_1$. Alpha reacts with all the sub-groups of A while $alpha_1$ reacts only with the sub-groups of A_1 and A_1B. To test for the sub-groups, $alpha_1$ is obtained free from alpha by absorbing anti-A serum with a suitable quantity of A_2 cells. The sera of 1–2% of A_2 persons and of approximately 25% of A_2B persons contain the agglutinin $alpha_1$. There is a further agglutinin, anti-O ($alpha_2$), which reacts with all O cells and most A_2 cells. This is found very occasionally in A_1, B, and A_1B sera. A table illustrates the groups and sub-groups with their usually occurring serum agglutinins and possible extra agglutinins.

Methods

(1) *Tube Method.* 0·08 cm.³ of high titre anti-A serum, diluted with an equal amount of normal saline, and a similar quantity of diluted high titre anti-B serum are delivered by graduated Pasteur pipettes into test tubes $2'' \times \frac{1}{4}''$ [approximately 5·0 × 0·6 cm.]. 0·08 cm.³ of a 5% suspension of the unknown blood in 3% sodium citrate is added to each tube. With suitable racks and a standard arrangement of the tubes, large numbers of tests can be carried out simultaneously. As controls, cells of known groups A, B and O should be tested in the same way. The tubes are shaken and left at room temperature for 2 hours. The contents of every tube which does not then show undoubted agglutinates on flicking with the finger are examined microscopically. The agglutinins in the serum are tested for by a similar technique, using known A_1 and B cells. It is important in these tests to look for weak "A" reactions with anti-A serum and to realise that the $alpha_1$ agglutinin can occur in the sera of groups A_2 and A_2B.

(2) *Tile Method.* On a warmed flat white opal-glass tile 0·05 cm.³ of high titre anti-B serum is placed in the left-hand space and an equal amount of high titre anti-A serum in the right-hand space; to each of these is added 0·05 cm.³ of the red cell suspension. The tile is rocked gently to ensure mixing and left to stand for 10–15 minutes. On agitation, agglutination, if present, can be easily seen against the white background. Unknown sera are similarly tested with known cells. Instead of using diluted blood, undiluted blood can also be used, provided that the amount of blood added is sufficient to colour the mixture only a light pink. With this technique the reading should be made in 2–4 minutes. Pseudo-agglutination may occur after this time.

Recommendation 1. The tube method is the most reliable, but where this is found impracticable a tile technique, using diluted blood, is recommended. Undiluted blood should not be used unless the workers have had long experience of the technique.

Recommendation 2. In the case of blood issued for transfusion cells should be examined for an agglutinogens serum for agglutinins. The double check is essential.

Direct Matching

Direct matching of the donor's cells against the recipient's serum before transfusion is extremely important. It should be omitted only in an acute emergency. The technique recommended is fundamentally the same as that already described.

Essential Properties of Test Sera

Serum to be used for blood grouping must fulfil certain conditions. (i) High titre. Only high titre sera, preferably

checked against the *Medical Research Council's* standard, should be used. The standards are available in the dried form. (ii) Capacity to react with A_2 and A_2B cells. Anti-A serum should be titrated in order to determine its capacity to react with A_2B cells. (iii) Absence of cold agglutinins. (iv) Absence of a tendency to cause rouleaux formation. (v) Freedom from fat.

Storage of Test Sera

(i) Temperature: test sera should be kept, if possible, frozen solid. If kept thus their strength is preserved almost indefinitely. If kept at 2–4° C. their strength is preserved for a variable period. (ii) Bulk: serum should be stored in bottles or ampoules which contain amounts suited to the particular needs of the workers who are to use them. A frequent small issue of supplies is more satisfactory than a single large issue. (iii) Asepsis: sterility is important but no antiseptic should be added to test sera. (iv) Dried grouping serum: completely dried serum efficiently sealed in ampoules under nitrogen will keep indefinitely without refrigeration.

Possible Sources of Error in Blood Group Determinations

(*a*) Technical: false negative results may be due to (i) failure to use high titre sera, (ii) failure to use anti-A serum capable of reacting with A_2 and A_2B cells, (iii) failure to recognize the time factor, (iv) the use of infected serum. False positive results may be due to (i) pseudo-agglutination or rouleaux formation, (ii) cold agglutination, (iii) the use of infected cell suspensions, (iv) the use of infected serum. (*b*) Clerical errors: these can be avoided only by careful checking at every stage by everyone concerned.

The Rh Factor

Human erythrocytes may contain in addition to the A and B agglutinogens a variety of antigenic components, of which the Rh factor is the most important. The 15% of English white subjects whose erythrocytes lack this factor are liable to form an agglutinin against this agglutinogen if it is introduced into their circulation. This may occur in pregnancy or with transfusions. Ideally, Rh-negative persons should be transfused only with Rh-negative blood. At present, however, this ideal may be regarded as impracticable.

Tests for Rh Agglutinogens and Agglutinins

Sources of test sera: (i) An animal, preferably the guinea-pig, may be immunized by being given a course of injections of blood from the monkey, *Macacus rhesus*. (ii) Serum may be obtained from a human being who has become immunized to the Rh antigen. Animal sera have to be absorbed [freed from other antibodies acting upon human erythrocytes] but they can be produced at will. With human sera a comparatively large amount can be obtained with little trouble, but may of course contain anti-A or anti-B agglutinins. If necessary, these human sera can be absorbed with A or B Rh-negative blood or neutralized with the appropriate human salivæ [which contain the antigens].

Method

One volume of 2% suspension of blood is placed with one volume of the test serum in a small tube 7 mm. in diameter. It is desirable to test cells with not less than three different sera. At the same time known Rh-positive and Rh-negative cells should be tested against the same sera to act as controls. The tubes should be left for at least one hour and preferably 2 hours in a water bath or incubator at 37° C. The sediments are then examined with a hand lens. When the reaction is negative the sediment is found to be homogeneous and to have a clear-cut circular edge. When the reaction is positive the sediment appears granular and the edge is serrated. When the tubes have been thus examined a little of the sediment in each tube is *gently* withdrawn by means of a Pasteur pipette and *gently* spread on a slide. Agglutinates are seen with those bloods giving a positive reaction. In case of discrepancy between the macroscopic and microscopic appearances it is advisable to rely upon the microscopic finding.

In testing unknown serum for the presence of anti-Rh agglutinins a similar procedure is followed using two known Rh-positive and two known Rh-negative bloods as the test cells.

Some sera containing anti-Rh agglutinins exhibit "zoning." It may therefore be necessary to test serial dilutions of the serum against known Rh-positive bloods.

Recommendations. (i) Mothers of infants manifesting signs of *erythroblastosis fœtalis* should not be transfused with blood unless known Rh-negative blood of suitable ABO group is available. In cases of urgency, plasma or serum may be used instead of whole blood. (ii) Recipients of either sex who are suspected of having become immunized to the Rh-factor should receive no further whole blood transfusions unless Rh-negative blood of suitable ABO group is available. (iii) When transfusions are given to infants affected with *erythroblastosis fœtalis*, blood from a group O Rh-negative donor should be used whenever possible.

Training of Personnel

All blood group determinations should be entrusted only to personnel who have had a sufficient period of training in a laboratory accustomed to such work. The necessity for employing trained personnel is emphasized since there has hitherto been a tendency to consider that anyone can be taught to determine blood groups in a few minutes. It is essential if errors are to be avoided that workers who are making blood group determinations should fully understand the underlying principles of the method.

[*Any workers on blood transfusion who would find the original 17-page Memorandum of value are invited to apply for a copy.*]

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TESTS FOR THE Rh FACTOR AND ITS ANTIBODY

by G. L. Taylor, *Proceedings of the Royal Society of Medicine*, **36**, 225–226, March 1943

This paper, which reports work done by the author on behalf of the *Medical Research Council* at the *Galton Laboratory* Serum Unit at Cambridge, cannot be much abbreviated and is reproduced below with only a few modifications and omissions.

The presence of Rh antigen in the erythrocytes is detected by agglutination on addition of serum containing the corresponding anti-Rh agglutinin. Grouping sera are obtained (i) from the rabbit or guinea-pig injected with rhesus monkey erythrocytes; (ii) from mothers of erythroblastic infants or from persons who have had hæmolytic transfusion reactions due to Rh.

The preparation of animal sera is difficult. Few human sera are strong enough, but they will, in the author's opinion, probably prove to be the most convenient, and will be prepared from the mothers of erythroblastic babies.

Detecting anti-Rh. Serum is heated for fifteen minutes at 56° C., and is then mixed with cells from at least two strongly positive donors, with negative cells, and with cells from the donor of the serum; some sera agglutinate their own cells and this is a necessary control. Known positive and negative cells should be of group O to avoid the possibility of reactions due to the A–B–O system of groups. Most grouping is done on a slide, tile or plate, but for Rh work tubes must be used. The author's tubes are 2 inches long by ¼ inch in diameter [approximately 5·0 × 0·6 cm.], and in them a series of falling dilutions of serum is prepared. In the first tube the serum is undiluted; in the second diluted 1 : 2; in the third 1 : 4 and so on for 6 or 7 tubes. To each tube is added a volume of erythrocyte suspension (1 to 2% whole blood) of a strongly positive donor. Cells and serum are mixed by picking up a tube and flicking with the finger, and on each tube is placed a glass cap to prevent evaporation and serve as place-marker in reading tests. To other identical series are added cells from a second strongly positive person, negative cells, and the cells of the donor of the serum. If the donor is the mother of an erythroblastic baby, if the A–B–O groups permit, and if the cells are available, the serum is also titrated with cells from the baby and the father. The tests are stored at room temperature and an identical set in the incubator at 37° C. Some human anti-Rh sera work better at room, others at body temperature. The great majority are better at body than at room temperature.

After an hour, or better after two, the tests are read. The character of the sediment at the bottom of the tube gives a good idea as to whether there is clumping or not. The cap is removed and the sediment examined with a hand lens, but the final diagnosis is based on microscopic examination. The greatest care and gentleness are needed in transferring some of the sediment to a microscope slide; any roughness may undo some of the more weakly positive reactions and a false negative may be recorded. Good reactions are easily seen; weakly reacting cells may cause trouble. In sedimenting, cells aggregate, and a beginner may mistake for

true agglutination a lumpy aggregation of cells; such an aggregation gradually breaks up. The author recommends this procedure because (i) titration indicates the strength of any antibody present, (ii) one serum, when undiluted, failed to react with some positive cells but, in titration, gave definite reactions in some dilutions.

When tests suggest that anti-Rh is present, the serum is mixed with positive cells from four or five other donors and with one or two lots of negative cells, and if it reacts with all or nearly all the positives and not with any negatives, it seems certain that anti-Rh is present. Indeed, when all the positives react and the negatives fail to, a simple sum gives exactly the odds that the serum contains anti-Rh. The author advises this titration technique in direct compatibility tests between recipient's serum and potential donors' cells.

With good sera, erythrocytes can be grouped for Rh by methods similar to those described. Titration is not needed and a serum which required titration would not be used. A volume of serum is mixed with a volume of erythrocytes and to avoid any tendency to give false agglutination, a volume of saline is added. Tests are stored at room or incubator temperature according to which is better for the sera in use. Bringing together antibodies and antigens of the A–B–O groups should be avoided, otherwise the Rh grouping cannot be done. Anti-A or anti-B in a serum can be absorbed by mixture with appropriate cells, *e.g.* A Rh-negative cells will remove anti-A and leave anti-Rh. Mixing the serum with the saliva of a person who secretes the appropriate antigen will also remove the A–B–O antibodies. About 80 % of people secrete in the saliva the A-B-O antigens present in their red cells. If a supply of Rh sera from all the four blood groups is available, there is no need for absorption.

No cells should be diagnosed as negative unless they have been tested with at least three strong anti-Rh sera. Some sera react well with all but a few Rh-positive cells. One may react well with the cells of *x* and poorly or not at all with those of *y*, whilst another does well with *y* and badly with *x*. If a serum tends to give slight false positive reactions, reference to the reactions given by other sera will help diagnosis.

Rh testing is difficult and needs considerable experience, but it will become less of an art and more of a science when good supplies of really strong sera are available, and these must come from the clinicians who are in charge of the mothers of erythroblastic babies.

In the author's unit, blood from 49 mothers of erythroblastic babies has been examined; 43 were Rh-negative, and in the sera of 37 of these, anti-Rh was found. In a random sample of 49 women only 7 or 8 would be expected to be Rh-negative. There can be no doubt that the Rh factor plays an essential part in the causation of most cases of *erythroblastosis fœtalis*.

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THE INVESTIGATION OF HÆMOLYTIC TRANSFUSION REACTIONS

by P. L. Mollison, *British Medical Journal*, **1**, 529–532 & 559–561, 1/5/43 & 8/5/43

This paper is a Report to the *Medical Research Council* from the South-West London Blood Supply Depot.

A hæmolytic transfusion reaction may be defined as the occurrence of an abnormal destruction of the red cells of either the donor or the recipient immediately after transfusion.

Following a normal compatible transfusion of fresh blood, the donor's erythrocytes are destroyed only at a slow rate. Approximately 1 % are eliminated each day. When incompatible blood is transfused, however, the donor's erythrocytes are rapidly destroyed. A similar rapid destruction occurs when blood which has been stored for an excessive length of time is transfused. Destruction of some of the recipient's own red cells may follow the transfusion of blood, the plasma of which contains high titre agglutinins incompatible with the recipient's red cells.

When transfusions are given to persons affected with certain varieties of hæmolytic anæmia the signs of hæmolysis may temporarily become more pronounced. Such reactions may not be due to any of the causes enumerated above.

The establishment of the diagnosis of a hæmolytic transfusion reaction depends (*a*) on the demonstration of increased blood destruction and (*b*) on the demonstration of the cause.

Signs of Increased Blood Destruction

Intravascular destruction results in the liberation of hæmoglobin into the plasma. This hæmoglobin may be eliminated in three different ways (Fairley, 1940): (i) It may be absorbed by the reticulo-endothelial system and converted to bilirubin. (ii) The hæmoglobin is broken down intravascularly. Hæmatin is formed and this combines with serum albumin to form methæmalbumin. (iii) Some hæmoglobin is excreted by the kidney, but only when the concentration in the plasma exceeds approximately 135 to 180 mg. per 100 cm.^{3} (Gilligan, Altschule & Katersky, 1941). When the urine is alkaline, most of the hæmoglobin will be in the form of oxyhæmoglobin. If the urine is neutral or acid, methæmoglobin will be formed. Hæmoglobinuria is always accompanied by albuminuria and sometimes by glycosuria. Some degree of renal failure may occur.

Extravascular destruction of erythrocytes results in the production of bilirubin. The degree of bilirubinæmia depends upon the rate of blood destruction and upon the efficiency of the liver in removing the bilirubin from the bloodstream.

When, as the result of intravascular or extravascular destruction, the serum bilirubin concentration exceeds approximately 4 mg. per 100 cm.^{3}, the recipient becomes jaundiced. Slow blood destruction is less likely to produce jaundice. The liver function may be impaired by the transfusion of incompatible blood. Evidence of liver damage is sometimes apparent at necropsy and may be manifested during life by the development of signs of toxic jaundice.

Because of the blood destruction, the increase in hæmoglobin concentration expected as a result of transfusion does not occur when there is a hæmolytic reaction.

Apart from the signs of increased blood destruction, the recipient may show signs of constitutional disturbance. A rise in temperature, with or without a rigor, is common. Severe lumbar pain and other symptoms, such as burning of the face, tightness of the chest, *etc.*, may occur. Some degree of collapse is common. These symptoms and signs may be delayed or may be entirely absent. Conversely, they may be produced by the transfusion of apparently compatible serum. There is an impression that the sequelæ of transfusion of incompatible blood are less severe now than formerly. This may be due to the increasing tendency to administer blood at a slow drip rate.

Destruction of Incompatible Blood

In vitro, incompatible red cells may be either agglutinated or hæmolysed by the recipient's plasma. *In vivo*, intravascular hæmolysis may occur, when only agglutination occurs *in vitro*. This is especially the case when the incompatibility involves the Rh factor.

After the transfusion of incompatible blood, clumps of agglutinated cells may be found in blood samples withdrawn from the recipient. These agglutinates may persist for at least 48 hours after transfusion. When the recipient has, at the time of the transfusion, plasma agglutinins incompatible with the donor's erythrocytes, the majority of the donor cells are eliminated within a few hours of transfusion. In such cases the recipient's plasma agglutinins undergo characteristic changes in titre (Rö, 1937; Wiener, Oremland, Hyman & Samwick, 1941; Mollison & Young, 1941). There is usually an initial reduction in titre followed by a rapid increase which reaches its peak 10–20 days after transfusion. When the recipient's plasma does not actually contain incompatible agglutinins, but there is a potential incompatibility as when Rh-positive blood is transfused to an Rh-negative recipient, the rate of elimination is variable. If the recipient fails to react to the stimulus of the foreign agglutinogen of the donor blood, the latter may survive perfectly normally. If the recipient does respond by producing anti-Rh agglutinins, the total survival of the donor cells is reduced. The term "inapparent hæmolysis" has been applied by Wiener to such cases of slow destruction after transfusion.

Destruction of Stored Blood

The rate of destruction of the erythrocytes of stored blood in the circulation of a normal recipient after transfusion depends upon (i) the length of storage of the blood, (ii) the preservative solution used, and (iii) the temperature of the storage. The erythrocytes of blood stored at 5° C. for periods up to 21 days in a citrate-glucose diluent (*M.R.C. War Memorandum* No. 1, 1940) survive well during the 24 hours after transfusion. Erythrocytes of blood stored

in a simple solution of sodium citrate, however, survive far less well, and after 17 days of storage are almost completely eliminated from the recipient's circulation within 24 hours of transfusion (Mollison & Young, 1942).

Destruction of Recipient's Red Cells by High-titre Incompatible Agglutinins

Intravascular agglutination and hæmolysis followed by bilirubinæmia have been produced experimentally by the injection of plasma containing high-titre incompatible agglutinins (Aubert, Boorman, Dodd & Loutit, 1942).

Hæmolytic Reactions in Persons affected with Hæmolytic Anæmias

An illustration of such reactions is seen in a case reported by Dacie & Firth (1943). The transfusion of agglutinin-free plasma resulted in hæmoglobinæmia. The transfusion of a concentrated suspension of red cells brought about a violent hæmolytic reaction, although the donor cells survived normally.

Hæmolytic Reactions simulated by the Transfusion of Hæmolysed Blood

Blood may become hæmolysed by the action of contaminating organisms, by being overheated, or by being stored for excessive periods under unsuitable conditions. The transfusion of such blood may give rise to phenomena indistinguishable from true hæmolytic reactions as defined above.

Investigation of Hæmolytic Reaction

The investigation is greatly facilitated if certain samples are available. These are (i) a sample of the donor's blood, (ii) a pre-transfusion sample of the recipient's blood, (iii) a sterile sample of blood from the recipient after transfusion, and (iv) a clean sample of urine. The third sample should be partitioned into two plain tubes (one sterile), and one tube containing dry oxalate.

Examination of the Samples

In any case some or all of the following tests may have to be made :

(*a*) *Donor's blood.* (i) Bacteriological examination ; (ii) estimation of free hæmoglobin in the supernatant plasma ; (iii) regrouping, testing both cells and plasma ; if necessary, Rh typing ; (iv) cross-matching of the cells against the recipient's pretransfusion serum sample ; (v) the titre of the agglutinins in the donor's plasma.

(*b*) *Recipient's blood.* (i) Bacteriological examination of the sterile sample ; (ii) estimation of free serum hæmoglobin and serum bilirubin ; examination for methæmalbumin ; (iii) serological tests—a 2% suspension in isotonic citrate of the oxalated sample is examined for the presence of free agglutinates ; regrouping, testing both cells and plasma and, if necessary, Rh typing and examination of the plasma for anti-Rh agglutinins ; titration of the agglutinins in the plasma for comparison with the results of titrations on later samples ; if necessary, differential agglutination tests to determine the proportions of recipient and donor blood.

(*c*) *Urine.* (i) Routine tests ; (ii) special tests for pigments, pigment casts and urobilin.

Preliminary regrouping of the blood of the donor and recipient may reveal a mistake in ABO grouping. In such cases, four of which are described in the original paper, no other tests will be necessary. Rh typing and cross-matching may reveal incompatibility, and, here also, four illustrative cases are described. Another case instanced is that in which a hæmolytic reaction was simulated by the transfusion of hæmolysed blood ; in a further case, there was a fatal reaction due to the transfusion of infected blood.

Two other cases of which particulars are given by the author show that transfusion reactions, thought to be due to hæmolysis of the donor blood, were actually not so, as the survival of the donor's erythrocytes was proved by differential agglutination.

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[1][*See BMB* **89**]

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I. THE MECHANISM OF RED BLOOD CELL DESTRUCTION

by B. G. Maegraith, N. H. Martin & G. M. Findlay, *British Journal of Experimental Pathology*, **24**, 58–65, April 1943

II. LYTIC AGENT AND INHIBITORY FACTORS IN HUMAN TISSUE AND SERA

by B. G. Maegraith, G. M. Findlay & N. H. Martin, *Lancet*, **1**, 573–575, 8/5/43

I. These two papers mark an advance of fundamental importance in the study of hæmatology. The precise mechanism by which red blood cells are destroyed in the body has not previously been described, although it has been vaguely assigned to the reticulo-endothelial system. It is now shown to be due to a specific enzyme present in the tissues of many organs. The activity of this enzyme is normally controlled by the presence in the serum, and possibly also in the tissues, of an inhibitory substance. This inhibitor, unlike the lytic enzyme, is not species-specific. The rate of erythrocyte destruction in the body is therefore controlled by the balance between these two substances.

The lysin present in the tissues was demonstrated by incubating suspensions of washed erythrocytes in buffered glucose-saline with small washed slices of the tissue concerned. After the lapse of 12 hours or more, hæmolysis began to occur and was maximal between the 18th and the 24th hour. In controls without a tissue slice, or with tissue which had previously been heated to 80° C. for 5 minutes, there was no lysis. When these experiments were repeated with serum present as well in a dilution of 1 : 100, lysis did not occur. An inhibitory substance was therefore present from the serum. Titration of this inhibitor showed that it was effective at high dilution, in some cases even up to 1 : 1280. Experiments on these lines were done with the blood and tissues of guinea-pigs, monkeys and man. In each case similar results were obtained. When, however, the tissues of one species were incubated with red cells of another species there was no hæmolysis. The lytic enzyme is therefore species-specific. Serum from any of the animals under test would, however, prevent lysis in the tissues and erythrocyte systems of any of the other animals. This showed that the inhibitor was not species-specific. The enzymatic nature of the lysin was inferred: from the time relations of the reaction, from its inactivation by gentle heat, and from the fact that its action could also be prevented by minute traces of mercuric chloride or potassium cyanide, both of which are well-known enzymatic poisons. The final proof of the enzymatic nature is still lacking, as the authors themselves are careful to point out, but there seems to be little doubt that this will be forthcoming when the experiments can be repeated and extended in a laboratory where the necessary facilities are available [the work now reported was carried out in a military laboratory overseas].

II. In the second paper the authors describe how the technique of demonstrating this inhibitor in serum was used to investigate some cases of blackwater fever. During the stage of acute hæmolysis, a substantial reduction in the titre of the inhibitor was found. On recovery the titre rose again to normal values.

In their discussion of these results the authors suggest " that at least one of the factors involved in the development of the abnormal degree of lysis in blackwater fever is a reduction in the activity of the circulating inhibitor, with the result that in blackwater fever the balance between the tissue lytic agent and the inhibitory factor is upset in the direction of lysis. This amounts to saying that in blackwater fever there exists no abnormal lysin ; rather the lysis is simply a manifestation of excessive uninhibited activity of a normal lytic process."

The technique described in these papers should be of use in future investigations into all the hæmolytic anæmias and may well result in a substantial increase in our knowledge of these obscure and fascinating diseases.

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SURVIVAL OF NORMAL ERYTHROCYTES AFTER TRANSFUSION TO PATIENTS WITH FAMILIAL HÆMOLYTIC ANÆMIA (ACHOLURIC JAUNDICE)

by J. V. Dacie & P. L. Mollison, *Lancet*, **1**, 550–552, 1/5/43

Very few attempts have so far been made to estimate the survival time of transfused blood in cases of familial hæmolytic anæmia, although important evidence might thus be obtained as to the mechanism of the hæmolytic process in this disease. A method of estimating the survival of transfused erythrocytes by means of differential agglutination was first applied to man by Ashby (1919), who showed that after transfusing blood of group O to a recipient of another group, say A, the recipient's cells could be agglutinated with anti-A sera and the unagglutinated group O cells (of the donor) could then be counted. Wiener (1934) and Mollison & Young (1942) both found that after transfusion the donor erythrocytes are steadily eliminated from the (presumably "normal") recipient's circulation over a period of 80–120 days, the average being 109 days. Various workers have estimated the survival of transfused blood in isolated cases of hæmolytic anæmia, but lack of certainty as regards the diagnosis, or technical difficulties, have marred their observations in most cases.

Lloyd (1941), basing his estimates on a study of the cell-diameter distribution curves after transfusion, found that in one of 2 patients afflicted with familial hæmolytic anæmia the transfused cells seemed to have disappeared by the fourth week and in the second case they survived for at least 17 days after transfusion. In cases in which the donor cells are of normal diameter and those of the recipient vary widely in diameter, as in the disease in question, it seems difficult accurately to study, by this method, the survival of the normal cells after a large proportion of them has disappeared. A modified Ashby's technique which, being independent of morphological differences, does not suffer from this disadvantage, was used by the present authors, from the Central Pathological Laboratory, Sector IX, and the South-West London Blood Supply Depot respectively, to estimate survival time in 6 cases. Five of these (cases 2, 3, 4, 5 and 6, aged 72, 45, 43, 41 and 9 years) with a family history of the disease, had suffered from anæmia and jaundice for many years, while in the sixth (case 1), an infant aged 1 year, the disease had been manifest since the age of 2 months.

In all 6 cases group O blood was used for transfusion (in case 3 the recipient belonged to group O and in the other cases to group A), and blood samples were withdrawn from the recipient before and immediately after the transfusion, on the following day, and then at intervals (usually every 14 days) until the experiments were terminated. Cases 2–6 received a concentrated cell suspension prepared from two or more bottles of blood stored in citrate-glucose for a few days (according to Mollison & Young, 1942, the survival of such blood in healthy subjects should be normal), and case 1 was given defibrinated blood previously stored for 24 hours. Since the use of large amounts of blood for transfusion greatly increases the accuracy of the Ashby method an initial concentration of between 800,000 and 2,200,000 donor cells per $mm.^3$ was produced in the recipient's circulation.

Ashby's original technique has proved unsatisfactory even with the high titre sera necessary to obtain complete agglutination of the recipient's erythrocytes, and various modifications can be employed with advantage (Mollison & Young, 1940). With a view to shortening the time necessary for full agglutination, and to improving agglutination without impairing the accuracy of the method, in these experiments the mixture of cells and serum was centrifuged at approximately 1500 revolutions per minute for 1 minute. The test-tubes containing the mixture were agitated to break up the sediment and free the unagglutinated cells, centrifuged once more, and again shaken. With anti-M and anti-N sera centrifugation cannot be employed because it usually causes some non-specific agglutination unless the sera have been more fully absorbed than usual. In such cases, therefore, the mixtures of cells and serum are allowed to stand for 2 hours in small flat-bottomed bottles of such a size that the mixtures are spread out in a thin layer.

In 5 out of the 6 patients with familial hæmolytic anæmia the rate of disappearance of the donor cells was normal and the total survival time was between 100 and 130 days. In case 3, however, destruction was more rapid, and was complete in about 60 days. Further study revealed that this patient was Rh-negative (the other 5 were Rh-positive), whereas the donor blood was Rh-positive and, following its disappearance, anti-Rh agglutinins appeared in the patient's serum.

A series of subsidiary experiments in which the survival time of blood taken from case 4 and transfused into patients with mild secondary anæmias was estimated by the method of Mollison & Young (1940), showed that blood taken before splenectomy was completely eliminated within 14 days of transfusion, while normal blood given to the same recipient at the same time survived normally. Blood taken from case 4 a year after splenectomy again rapidly disappeared from the circulation of a normal recipient after transfusion, only 32 % of the donor cells being present at the end of 8 days, while destruction was complete within 19 days.

The authors conclude that these observations support the theory that the basic abnormality in familial hæmolytic anæmia is the formation of erythrocytes with an increased tendency to hæmolysis and cannot be reconciled with any hypothesis assigning a major role to abnormal destructive mechanisms.

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BLOOD TRANSFUSION IN NOCTURNAL HÆMOGLOBINURIA

by J. V. Dacie & D. Firth, *British Medical Journal*, **1**, 626–628, 22/5/43

Chronic hæmolytic anæmia with nocturnal hæmoglobinuria (the Marchiafava-Micheli disease or "nocturnal hæmoglobinuria") is an uncommon type of hæmolytic anæmia for which there is no specific treatment and which, although variable in cause and severity, ultimately proves fatal. It seemed possible from the observations of Ham (1939), who reported remissions after transfusion in spite of an initial severe hæmolytic reaction, that blood transfusion might have an inhibitory effect on hæmolysis, perhaps owing to the presence of a naturally occurring antihæmolytic substance in the transfused blood (Josephs, 1938). The present authors, a Pathologist in the *Emergency Medical Service* and the Senior Physician to *King's College Hospital*, London, respectively, record the results of an experiment to determine whether this hypothetical inhibitory effect might prove to be a basis for an effective line of treatment.

The subject of the experiment, a thirty-eight year old woman who had suffered from anæmia and abnormal discoloration of the urine passed in the early morning since November 1940, was admitted to hospital with jaundice on July 1st, 1942. The tentative clinical diagnosis of nocturnal hæmoglobinuria was confirmed by serological tests which revealed: (i) that a proportion of the patient's cells were lysed *in vitro* after 1 hour's incubation at 37° C. in autogenous fresh serum or that of normal subjects of the same group; (ii) that lysis was sensitive to changes in pH, and was increased by the addition of fresh guinea-pig serum from which all anti-human heterolysin had been absorbed; (iii) that the patient's cells were not lysed by guinea-pig serum in the absence of human serum; and (iv) that normal cells were not lysed by the patient's serum under any conditions.

After 3 weeks' observation, therefore, 2 litres of stored (6 weeks-old) filtered human serum were administered intravenously within 12 days. After each bottle of serum, gross hæmoglobinæmia and hæmoglobinuria occurred, followed by a period of freedom only interrupted by administration of a further bottle. The urine remained clear for 10 days after the last bottle of serum was given on September 1st, but laboratory investigations revealed no significant changes in Hb or in erythrocyte number per $mm.^3$, and a standard *in vitro* test showed no alteration in the sensi-

tivity of the patient's cells to hæmolysis by her own serum. No evidence was thus obtained to support the hypothesis that an antihæmolytic factor was lacking. On September 11th the patient was given 500 cm.³ of a concentrated cell suspension of Group O citrate-glucose blood within 90 minutes. Before the transfusion had been quite completed symptoms of a hæmolytic reaction began to be felt. These grew more severe during the following night, but subsided within 24 hours and by September 15th the specimens of urine passed were clear. The period of remission which ensued lasted for 6 weeks. Laboratory investigations showed that during the hæmolytic episode more than half of the patient's own erythrocytes were destroyed while the survival of the transfused blood was unimpaired. The ultimate rise in blood count, which coincided with the remission from hæmoglobinuria, was due to the good survival of the transfused cells, for the patient's own cells did not rise in number above the pre-transfusion level.

The authors conclude that these observations support the hypothesis that the basis of this strange disease is an undefined abnormality of the patient's erythrocytes which causes them to hæmolyse in the presence of human serum both *in vivo* and *in vitro* (*see* Ham & Dingle, 1939). The possibility that the patient's erythrocytes were sensitised by the presence of an immune body or other hæmolytic agent would appear to be negatived by the unimpaired survival of the transfused normal cells.

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FAMILIAL HÆMOLYTIC ANÆMIA (ACHOLURIC JAUNDICE), WITH PARTICULAR REFERENCE TO CHANGES IN FRAGILITY PRODUCED BY SPLENECTOMY

by J. V. Dacie, *Quarterly Journal of Medicine*, **12**, 101–118, April 1943

The nature of familial hæmolytic anæmia, or acholuric jaundice as it is sometimes called, is still obscure, but it is to be hoped that the increasing use of exact methods of research, such as have been applied to it in 24 cases reported by the author of this paper, will help to solve the problem.

The criteria that he accepts for diagnosis are:

"An anæmia of hæmolytic type with increased susceptibility of the erythrocytes to hæmolysis by hypotonic saline, spherocytosis, an abnormal tendency of the blood to lysis *in vitro* on incubation at 37° C., and splenomegaly in which engorgement with blood is the outstanding feature. A history of recurrent anæmia or jaundice over a period of years and a positive family history have been taken as confirmatory, though not essential for diagnosis."

The technique for estimating fragility was devised by Creed (1938) and modified by Dacie & Vaughan (1938). Briefly it consists in preparing a series of tubes containing progressive dilutions of sodium chloride, each differing by 0·02%. Blood is added to each tube and the degree of hæmolysis that occurs is estimated colorimetrically. The results are plotted graphically. The normal curve is steep and smooth, hæmolysis beginning at about 0·5% NaCl and being complete at about 0·36%. Curves of this type were found in 5 of the 24 cases studied. In 12 cases a "tailed" curve was obtained. This is a curve which follows the normal closely in the lower dilution range, but is prolonged above 0·5%. Some hæmolysis may even be detected up to about 0·7% NaCl. A third type of curve, found in 6 cases, is called the "diagonal" type. This is almost a straight line, sloping evenly from about 0·7% to 0·4%; it nowhere approximates to the normal curve and presents a great contrast to it.

Splenectomy was performed on 12 cases, 7 of which had "tailed" curves before operation; 24 hours later there was a slight increase in the mean fragility, but this later fell to normal, and by the 10th day after operation the curves were nearly normal in shape. Two cases in which there were "diagonal" curves before splenectomy showed a decrease of fragility afterwards, but the curves, though altered, did not become absolutely normal.

The transient post-operative increase in fragility may have been due to the effects of the anæsthetic. The later decrease may have been due either to the removal of an organ which increased erythrocyte fragility, or to the production by the bone marrow of a more resistant type of erythrocyte as a result of the diminished rate of hæmolysis after splenectomy.

Histologically, the excised spleens all showed the well-known features of this disease: distension of the pulp with erythrocytes, small sinuses with endothelial nuclei often protruding into their lumina, absence of obvious erythrophagocytosis and of hyperplasia of the reticulum cells, presence of unusual amounts of iron-containing pigment and fibrosiderotic nodules.

Some experiments were done to try to find the cause of the engorgement. Perfusions, at 100 mm. Hg pressure, first with saline and then with suspensions of the easily recognisable fowl erythrocytes were done on some of these spleens as well as on some normal controls. The experiments showed that there was no obstruction to these cells in their passage through the direct pathways between capillaries and sinuses. Nevertheless it is more difficult to free the abnormal spleens of erythrocytes by saline perfusion than it is to wash the cells out of normal organs.

Spherocytosis, a term indicating the presence of erythrocytes smaller in diameter but of abnormal thickness, is a constant feature of this disease. It is not yet settled whether this represents a primary disturbance of erythropoiesis, or whether it results from the presence of an abnormal hæmolytic agent or mechanism. The author inclines to the former view after a discussion of the various hypotheses put forward by many other writers.

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EFFECT OF THE pH OF THE BLOOD ON HÆMOLYSIS: With Special Reference to Blackwater Fever

by F. Smith & R. W. Evans, *British Medical Journal*, **1**, 279–282, 6/3/43

This paper is by a physician and a pathologist now working with the British Army in a malarial district overseas. Sixteen cases of blackwater fever were studied with the object of finding the cause of the intravascular hæmolysis so that a logical remedy could be applied.

The authors first found that in normal people there is a wide variation in the erythrocyte fragility throughout the course of the day. In samples taken at hourly intervals for 24 hours the erythrocytes were more fragile at night than during the day. It was shown by Dacie, Israëls & Wilkinson (1938) that the pH of the blood is lower during sleep at night than it is in the daytime. The present authors also found that the erythrocyte fragility was much affected by the pH of the diluting fluid used. The more acid the fluid the more fragile the cells became. In patients with blackwater fever the erythrocytes were more fragile than in normal controls.

In all cases studied the fever started with a rigor and was preceded by a sense of intense fatigue for 2 or 3 days. Vomiting was also a constant feature. In this disease a man may lose the equivalent of 2 to 3 litres of blood by intravascular hæmolysis in 36 hours. The clinical picture therefore has much in common with "shock" and so has the treatment.

Sections of the kidney show blockage of the tubules. This used to be thought due to crystals of acid hæmatin but is more likely due to methæmoglobin and cellular debris. Treatment must aim at preventing or lessening the renal blockage. Five factors are considered with the treatment appropriate to each.

Factor A. The hæmolysis: The experiments already mentioned indicate that sufficient alkali must be given to raise the pH of the blood. This will render the cells less fragile and hæmolysis will cease. For rapid effects 20 cm.³ of a 2M or 3M solution of sodium lactate can be given intravenously every 8 hours. The solution can be sterilised by boiling, but is rather apt to cause thrombosis of the vein into which it is injected. Less rapid effects are obtained by giving by mouth every 2 hours 1·3 g. of sodium bicarbonate and 1·3 g. of sodium citrate in 15 cm.³ of water.

Factor B. The obstruction of the renal tubules by the products of hæmolysis: Sweating may account, in the Tropics,

for half the fluid output of the body. It leads to concentration of the urine and is therefore dangerous when the renal function is impaired. Tepid sponging should therefore be ordered when the temperature exceeds 103° F. [39·5° C.]. The fluid balance should be carefully recorded; provided that two-thirds of the intake is excreted by the kidneys the chance of recovery is good. Excess of fluid given intravenously is dangerous as it may lead to generalised œdema.

Factor C. The severe degree of anæmia, damaging the kidneys' excretory function: The erythrocyte count often falls to 2,000,000, while the leucocytes rise. Blood transfusion is often advocated, but the present authors advise caution in its use. Patients are liable to have exceptionally severe transfusion reactions, so that particular care in doing the compatibility tests is needed. Double cross-matching at room temperature and also at 4° C. for 24 hours should always be done. Even with blood that is quite compatible the transfusion should be started very slowly, only 20 drops being given in the first 10 minutes, after which the rate may be increased. Later, when the urine is clear, iron therapy should be started.

Factor D. The production of acid hæmatin crystals in the renal tubules: The high degree of alkalinisation will prevent this from occurring.

Factor E. The existence of living malarial parasites in the blood: The fact that the patient is still suffering from malaria should not be forgotten. Half a tablet of mepacrine (atebrin) should be given 48 hours after the hæmoglobinuria has ceased. If there are no ill effects from this, continue with half a tablet twice a day for a week and then two tablets every third or fourth day.

Summary: In this paper the claim is made that erythrocyte fragility varies from hour to hour and is related to the pH of the blood. The authors were unable to determine the pH of the samples tested for fragility. This was because the apparatus was not available, owing to service conditions, for performing the tests with sufficient accuracy. The good effect of massive alkaline therapy goes far to support their claim. Of the 16 patients seen, those receiving alkali did better than those not so treated.

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HÆMOGLOBINOMETRY AND THE USE OF THE HÆMATOCRIT: A Report to the Traumatic Shock Committee of the Medical Research Council

British Medical Journal, **1**, 209–212, 20/2/43

For some years interest has been renewed in the problem of "wound shock." With the development of blood transfusion technique on a large scale, and especially the introduction of plasma transfusion, great advances in the treatment of this condition were made. It was nevertheless apparent that much research was still needed and the *Medical Research Council* accordingly appointed a committee to investigate "traumatic shock." As this body soon found that progress was hindered by the lack of uniformity of methods for estimating the various constituents of the blood, a sub-committee was appointed to report on the methods in use. In the present report, the findings and recommendations of this sub-committee are set forth.

The complexity of hæmoglobin chemistry is first briefly considered. In normal venous blood, hæmoglobin exists mainly in two forms—oxyhæmoglobin (O_2–Hb) and reduced hæmoglobin (Hb). Up to 3 % of methæmoglobin (met–Hb), formed by the reduction of the ferrous iron in O_2–Hb or Hb, may also be found in normal blood. In city dwellers or heavy smokers up to 3 % of carboxyhæmoglobin (CO–Hb) may also be present; it is 210 times more stable than O_2–Hb and does not carry oxygen. In carbon monoxide poisoning 20 to 50 % of the total Hb may be CO–Hb. During treatment with certain drugs, especially sulphanilamide, both met–Hb and sulphæmoglobin (S–Hb) may be found in appreciable quantities. Neither of these compounds carries oxygen to the tissues. It is the simultaneous presence of several of these compounds that gives rise to the difficulties of hæmoglobin estimation.

There are five types of methods used for hæmoglobin estimation: (*a*) Those involving gas analysis are accurate in experienced hands but are not suitable for general use. They measure the oxygen capacity of the blood. (*b*) Colorimetric methods are those in which the colour developed in the test solution is compared with a standard. In (*c*) photo-metric and (*d*) photo-electric methods it is the amount of light passed by the solution that is estimated. Both types are subject to several sources of error of which the human factor is one. The error is eliminated in the photo-electric methods in which the amount of light transmitted is measured by a photo-electric cell. The cell itself, however, introduces other errors. The fifth type consists of (*e*) methods for estimating the iron content of well washed lysed red cells. The sub-committee regards this as the best check on all other methods and prefers the method of titration of ferric iron with titanous sulphate (Klumpp, 1934).

For all estimations, venous blood taken without stasis is recommended, although if it is necessary to use capillary blood the ear should be punctured rather than the finger.

As there was a lack of standardisation in the instruments commercially available the sub-committee sought the help of the *British Standards Institution*. Together they investigated and standardised the Haldane-Gowers hæmoglobinometer, a well-known and simple instrument in which CO–Hb is used. They recommend the use of instruments that conform to their specification B.S. No. 1079–1942. In this the forms of pipette, dilution tube and colour tube have been accurately defined. Instruments can be tested for conformity with this standard by the *National Physical Laboratory*. It is hoped that the adoption of this standard may lead to that uniformity which is essential if hæmatological results are to be compared. Where CO is not available the acid hæmatin method of Sahli should be used. The *British Standards Institution* propose to standardise this instrument too. The sub-committee emphasises that the standard now proposed is only an *interim* standard until a more satisfactory one, related to the total hæmoglobin in normal human blood, has been defined.

The hæmatocrit is used to determine both the relative proportion of cells to plasma and also the total volume of red cells in a known volume of blood. From this, knowing also the total red cell count, the mean corpuscular volume of the red cells may be calculated. The method of Wintrobe & Landsberg (1935) is recommended.

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[A full discussion of many of the points mentioned in the report summarised above is given by J. W. Clegg & R. G. Macfarlane (1941) in an unpublished *Medical Research Council Memorandum on the Estimation and Standardisation of Hæmoglobin.*]

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STANDARDISATION OF THE HALDANE HÆMOGLOBINOMETER

by R. Donaldson, H. G. W. Harding & G. Payling Wright,

Journal of Pathology and Bacteriology, **55**, 205–215, April 1943

The importance of standardisation of methods for estimating the hæmoglobin content of blood has recently been emphasised in a Report to the Traumatic Shock Committee of the *Medical Research Council* [*see BMB* **92**]. This paper, from the Light Department of the *National Physical Laboratory* and the Department of Pathology of *Guy's Hospital*, London, contains the technical details of preparing the new standard in strict conformity with Haldane's original specification (1900–01). New knowledge of hæmoglobin chemistry made it possible for the authors to lessen the possible sources of error in preparing their solution of carboxyhæmoglobin. The solution finally obtained was perfectly clear and has remained unaltered for four years.

As Haldane's method depends on a colour comparison, it seemed better to re-define the standard in terms of colour and to express the tolerances on the same scale.

It is well known that suitable mixtures of the three primary colours can produce any desired colour or tint. The standards necessary for measuring colours in this way were defined in 1931 by the *Commission Internationale de l'Eclairage*. Their reference colours are known as X, Y and

Z. On this system the colour of the standard tubes of carboxyhæmoglobin is expressed by the formula

$$0{\cdot}4805X + 0{\cdot}3432Y + 0{\cdot}1763Z$$

The limits of tolerance are found empirically to be ± 2% in carboxyhæmoglobin concentration.

One advantage in defining the standard in terms of colour is that any tube can be tested as it is and related accurately to the present standard. A suitable correction factor can then be applied to any results obtained with it. For comparisons of this sort the laborious technique used to obtain the colour formula need not be employed. A suitably sensitive optical comparator can be used instead.

An important feature of the specification issued by the British Standards Institution (B.S. 1079 : 1942) is the allowance for variations in tube diameters. In making up their standard solution, manufacturers will in future vary the dilution of the carboxyhæmoglobin solution to suit the diameter of the tube in which it is to be put. A wide tube will be filled with a more dilute solution than a narrow one and *vice versa*. The amount and colour of the light transmitted by the tubes will be the same. Similar variations are permitted in the graduated tubes in which the blood under test is diluted. Thus, although more latitude is allowed in the construction of the instruments, there will be a final gain in accuracy.

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STUDIES ON STORED BLOOD : I. Results in a Series of 427 Transfusions ; II. The Leucocytes in Stored Blood ; III. The Oxygen Capacity of Stored Blood ; IV. Modifications in the Equipment for Blood Transfusion ; V. Observations on the Coagulation Mechanism in Stored Blood ; VI. Changes in the Erythrocytes during Storage ; VII. The Effect of Sodium Sulphapyridine, Albucid Soluble and Hydrogen Ion Concentration on Phagocytosis ; VIII. The Effect of Transfusion on Capillary Resistance ; IX. Further Observations on the Effects of Storage on Erythrocytes ; X. Complement, Iso-Agglutinins and Agglutinogens

by A. Crosbie, J. W. Czekalowski, H. Scarborough, C. P. Stewart & J. C. Thompson, *Edinburgh Medical Journal* [*see references at foot of text*].

A series of papers from the Edinburgh *Emergency Blood Transfusion Service* has appeared since the outbreak of war. Paper I (Stewart, 1940a) deals with the organisation of the Service which was modified and expanded to form a " blood bank." The technical methods and apparatus used are described in this paper and in paper IV (Stewart, 1940b) of the series. A particularly interesting feature is the method of blood withdrawal which ensures that the blood is never in contact with an excess of citrate. The stream of blood from the donor's vein mixes with a stream of 3·8% sodium citrate delivered from a measured container holding 50 cm.³ The concentration of citrate in the final mixture (450 cm.³) is approximately 0·38%.

Paper II (Crosbie & Scarborough, 1940) of the series deals with changes in the leucocytes, 50% of which disappear from blood during 4–5 days of storage. This disappearance is not influenced by oxygen saturation or by maintaining or increasing the concentration of glucose. Lymphocytes disappear more slowly than polymorphonuclear neutrophils. Eosinophils and monocytes are intermediate. The motility of leucocytes is unimpaired during the first four days of storage but diminishes after that. It cannot be observed after the sixteenth day.

In paper VII (Czekalowski, 1941) it was shown that the phagocytic index and the percentage of leucocytes taking part in phagocytosis are both increased by sodium sulphapyridine in concentrations ranging from 55·3 to 0·33 mg./litre. This effect was analysed and the authors believe it to be due to a stimulating effect of the drug on leucocytes.

Papers VI (Crosbie & Scarborough, 1941) and IX (Crosbie & Scarborough, 1942a) reported studies on the effect of storage on erythrocytes. Considerable variation was noted between different samples of blood. The rate and degree of disappearance of erythrocytes was found to be dependent on the initial count, the fall in numbers being greater with higher counts. This fall in the erythrocyte count is associated with an increase in the corpuscular volume and thickness. Erythrocytes in various specimens of blood attain the spherical state at approximately the same rate, the time taken varying with the specimen from 3–10 days. The development of spherocytosis is accompanied by an increase in both osmotic and mechanical fragility and spontaneous hæmolysis occurs as early as the tenth day of storage. The erythrocyte sedimentation rate becomes gradually slower as a result of storage, an effect which can be induced in fresh erythrocytes by mixing them with stored plasma.

In paper III (Scarborough & Thompson, 1940) in the series it was shown that neither the hæmoglobin content nor the oxygen-capacity is impaired by storage for periods of up to 30 days.

In paper V (Crosbie, Scarborough & Thompson, 1941) the authors report their observations on the coagulation mechanisms in stored blood. They show that the thrombocytes agglutinate during the first 2–3 days and that their number is greatly reduced after 4–6 days. This disintegration is associated with increased coagulability of the plasma. The coagulation time (Howell) and prothrombin time (Quick) gradually increase, reaching 50% of the initial values in about 35 days. No change in the fibrinogen content of blood was found to occur during 50 days of storage.

In paper VII (Scarborough, 1941) an immediate and remarkable increase in capillary resistance is shown to follow transfusion of blood or plasma (but not saline). This effect has been demonstrated to follow the transfusion of fresh blood, stored blood, and plasma stored for 7 months.

In paper X (Crosbie & Scarborough, 1942b) of this series, the authors report observations on complement, iso-agglutinin and agglutinogen potencies. Hæmolytic Complement activity was assessed in citrated plasma in terms of the greatest dilution at which hæmolysis was observed after incubation at 37° C. for 40 minutes. A 2% suspension of washed erythrocytes in contact with a standard dilution (5 M.H.D.) of amboceptor was used as indicator. The complement titre was found to fall rapidly to 30–50% of its initial value during the first 2–4 days of storage (in contact with cells). The rate of decrease did not appear to be greater when the initial value was high.

The titre of the iso-agglutinins α and β was assessed by the usual technique in terms of the greatest dilution in normal saline at which agglutination could be observed with standard fresh erythrocytes. It was found that the agglutinin titre was maintained for at least 6–8 weeks of storage. After that a slight fall occurred. Agglutinogen potency of stored erythrocytes was assessed in a similar manner against standard high-titre serum. For periods up to 8 weeks the agglutinogen potency was not affected by storage.

In the course of the investigation it was found that blood samples kept at room temperature for short periods (2–7 days) gave a better agglutination reaction when the cells were diluted with 3·8% citrate instead of 0·9% saline. The authors accordingly suggest that 3·8% sodium citrate is to be preferred as a diluent to 0·9% sodium chloride for blood taken for grouping purposes if the tests cannot be performed at once.

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STUDIES ON STORED BLOOD : XI. Phagocytosis in Stored Citrated Blood and the Opsonic Power of Stored Liquid Plasma

by J. W. Czekalowski (Poland), *Edinburgh Medical Journal*, **50**, 40–56, January 1943

Although in recent years the value of stored erythrocytes as oxygen carriers, the persistence during storage of the factors concerned in blood coagulation, and the behaviour of the

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"defence mechanism" have all been the subject of numerous investigations, the literature on phagocytosis in stored blood, one of the elements of this mechanism, appears to be scanty. The findings of previous investigators as regards the effect of citrate on phagocytosis are conflicting, but Karavanoff (1935) and MacDonald & Stephen (1939) found a rapid decrease in phagocytic power as a result of storage. Kolmer (1939), using citrated (0·35%) blood, observed that phagocytosis for *staphylococcus aureus*, *streptococcus hæmolyticus* (Group A) and *B. coli* was in some cases slightly reduced after 24 hours' storage, more markedly reduced after 3 days, and disappeared after about 7 days.

The present author, working in the Edinburgh and South-East of Scotland *Emergency Blood Transfusion Service*, found (1942) that with blood stored in 0·38% sodium citrate, phagocytosis for *streptococcus viridans* (Type R) disappeared between the 6th and 8th days of storage. In the investigation now reported, total leucocyte count, differential count, viability of leucocytes, "index of degeneration" (number of degenerated forms expressed as a percentage of total) were assessed in blood withdrawn from middle-aged Group O donors and stored in 0·38% sodium citrate at 2·5° C. In a phagocytic system (the standard procedure was to mix equal volumes of the bacterial suspension and the appropriate plasma and incubate at 37° C. for 30 minutes with continuous shaking) containing *S. viridans* (type R) the number of cells taking part in phagocytosis and the mean number of ingested organisms per leucocyte were also determined. Degenerative changes (nuclear deformity and junction kariorrhexis) in the leucocytes were observed after storage for 24 hours to be followed within 72 hours by kariopynosis, kariolysis, cytoplasmic vacuolation, granularity and basophilism. The index of degeneration was found to be 19% 24 hours after withdrawal, and reached 95% after storage for 8 days. During the first 3–5 days of storage viability of the leucocytes was well maintained but was absent by about the 14th day. From the very beginning of storage there was a rapid decrease in phagocytic activity until the 3rd or 4th day, and phagocytic activity ceased after 5–8 days of storage. The addition of large amounts of stored plasma to the phagocytic system, or incubation for 60–90 minutes, had very little effect in prolonging phagocytosis.

In these experiments the cells and plasma were stored together for similar periods. The results suggest that the gradual decline in phagocytic activity was due not only to disappearance of viable and morphologically normal leucocytes but also to changes in the plasma. In a second series of experiments the stored cells were incubated with a mixture of bacterial suspension and fresh plasma. It was concluded that, as phagocytosis was now appreciable until the 11th or 12th day of storage, fresh plasma had a stronger opsonic activity than the stored plasma. It was also found that when phagocytosis had ceased in systems containing stored plasma, it could be restored by the addition of fresh plasma.

In a third series of experiments the phagocytic system contained fresh washed cells, bacterial suspension and stored plasma. In such a system phagocytosis remains appreciable for 20 days (instead of 5–6 days with stored cells); but with plasma stored for not more than 4 days there was no definite change in the percentage of cells taking part in phagocytosis.

The author concludes that decrease and final disappearance of phagocytosis from stored blood should be referred not only to degeneration and dissolution of leucocytes but also to the disappearance of certain factors from the plasma. One of these factors is a normal opsonin, but a second is either complement or one of its components or an unknown substance which stimulates opsonisation. The opsonising activity of stored plasma can be increased by the addition of a little fresh plasma to an extent greater than can be accounted for by the opsonising effect of the latter. It is suggested that during opsonisation a compound BO_x is formed between bacteria (B) and opsonin (O), x representing the "degree of opsonisation" and hence the tendency to be ingested. The formation of BO_x is potentiated by a second factor which may be complement or one of its components. Phagocytosis of the opsonised organism requires a "normal" leucocyte and involves a reaction between BO_x and active groups at the cell surface. In a series of systems in which the leucocytes are of constant phagocytic power this theory requires that phagocytosis will be determined by the number of opsonised organisms. Experimental evidence is cited in support of this hypothesis and further experiments to test it are planned.

A practical implication of this study is that blood stored for more than 24 or at the most 48 hours should not be transfused with the object of strengthening the recipient's "defence mechanism" in cases of infection and especially in septicæmias.

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This Bulletin is published by the British Council. Requests for further information about any of the investigations reported should be addressed to The British Council, Medical Department, 3 Hanover Street, London, W. 1, England

Printed by Spottiswoode, Ballantyne and Co. Ltd., Colchester, London and Eton